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FILE 'BIOSIS' ENTERED AT 11:36:52 ON 01 JUL 2004

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=> s aryl and flavanone and isoflavanone

L1 6 ARYL AND FLAVANONE AND ISOFLAVANONE

=> d 11 1-6 ti

L1 ANSWER 1 OF 6 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN

TI Flavonoid 6-hydroxylase from soybean (Glycine max L.), a novel plant P-450 monooxygenase.

L1 ANSWER 2 OF 6 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN

TI BIOSYNTHESIS OF PTEROCARPAN ISO FLAVAN AND COUMESTAN METABOLITES OF MEDICAGO-SATIVA CHALCONE ISO FLAVONE AND ISO ***FLAVANONE*** PRECURSORS.

L1 ANSWER 3 OF 6 EMBASE COPYRIGHT 2004 ELSEVIER INC. ALL RIGHTS RESERVED. on STN

TI Lipase-catalyzed chemo- and enantioselective acetylation of 2-alkyl/ ***aryl*** -3-hydroxypropiofenones.

L1 ANSWER 4 OF 6 EMBASE COPYRIGHT 2004 ELSEVIER INC. ALL RIGHTS RESERVED. on STN

TI Flavonoid 6-hydroxylase from soybean (Glycine max L.), a novel plant P-450 monooxygenase.

L1 ANSWER 5 OF 6 CAPLUS COPYRIGHT 2004 ACS on STN

TI Flavonoid 6-hydroxylase from soybean (Glycine max L.), a novel plant P-450 monooxygenase

L1 ANSWER 6 OF 6 CAPLUS COPYRIGHT 2004 ACS on STN

TI Cytochromes P450 involved in isoflavone biosynthesis of soybean and Medicago and the genes encoding them and their uses

=> d 1 2 3 6 ibib ab

L1 ANSWER 1 OF 6 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN

ACCESSION NUMBER: 2001:232219 BIOSIS

DOCUMENT NUMBER: PREV200100232219

TITLE: Flavonoid 6-hydroxylase from soybean (Glycine max L.), a novel plant P-450 monooxygenase.

AUTHOR(S): Latunde-Dada, Akinwunmi Olumide; Cabello-Hurtado, Francisco; Czittrich, Nikola; Didierjean, Luc; Schopfer, Christel; Hertkorn, Norbert; Werck-Reichhart, Daniele; Ebel, Juergen [Reprint author]
CORPORATE SOURCE: Botanisches Institut der Universitaet, Menzinger Strasse 67, D-80638, Muenchen, Germany
j.ebel@botanik.biologie.uni-muenchen.de
SOURCE: Journal of Biological Chemistry, (January 19, 2001) Vol. 276, No. 3, pp. 1688-1695. print.
CODEN: JBCHA3. ISSN: 0021-9258.
DOCUMENT TYPE: Article
LANGUAGE: English
ENTRY DATE: Entered STN: 16 May 2001
Last Updated on STN: 19 Feb 2002

AB Cytochrome P-450-dependent hydroxylases are typical enzymes for the modification of basic flavonoid skeletons. We show in this study that CYP71D9 cDNA, previously isolated from elicitor-induced soybean (*Glycine max* L.) cells, codes for a protein with a novel hydroxylase activity. When heterologously expressed in yeast, this protein bound various flavonoids with high affinity (1.6 to 52 μ M) and showed typical type I absorption spectra. These flavonoids were hydroxylated at position 6 of both resorcinol- and phloroglucinol-based A-rings. Flavonoid 6-hydroxylase (CYP71D9) catalyzed the conversion of ***flavanones*** more efficiently than flavones. Isoflavones were hardly hydroxylated. As soybean produces isoflavonoid constituents possessing 6,7-dihydroxy substitution patterns on ring A, the biosynthetic relationship of flavonoid 6-hydroxylase to isoflavonoid biosynthesis was investigated. Recombinant 2-hydroxy- ***isoflavanone*** synthase (CYP93C1v2) efficiently used 6,7,4'-trihydroxyflavanone as substrate. For its structural identification, the chemically labile reaction product was converted to 6,7,4'-trihydroxyisoflavone by acid treatment. The structures of the final reaction products for both enzymes were confirmed by NMR and mass spectrometry. Our results strongly support the conclusion that, in soybean, the 6-hydroxylation of the A-ring occurs before the 1,2-***aryl*** migration of the flavonoid B-ring during ***isoflavanone*** formation. This is the first identification of a flavonoid 6-hydroxylase cDNA from any plant species.

L1 ANSWER 2 OF 6 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN
ACCESSION NUMBER: 1979:241469 BIOSIS
DOCUMENT NUMBER: PREV197968043973; BA68:43973
TITLE: BIOSYNTHESIS OF PTEROCARPAN ISO FLAVAN AND COUMESTAN METABOLITES OF MEDICAGO-SATIVA CHALCONE ISO FLAVONE AND ISO ***FLAVANONE*** PRECURSORS.
AUTHOR(S): DEWICK P M [Reprint author]; MARTIN M
CORPORATE SOURCE: DEP PHARM, UNIV NOTTINGHAM NG7 2RD, ENGL, UK
SOURCE: Phytochemistry (Oxford), (1979) Vol. 18, No. 4, pp. 597-602.
CODEN: PYTCAS. ISSN: 0031-9422.
DOCUMENT TYPE: Article
FILE SEGMENT: BA
LANGUAGE: ENGLISH

AB Comparative feeding experiments in CuCl_2 - and UV-treated lucerne (*M. sativa*) seedlings showed that 2',4,4'-trihydroxychalcone-[carbonyl- ^{14}C] and formononetin-[Me- ^{14}C], but not 2',4'-dihydroxy-4-methoxychalcone-[carbonyl- ^{14}C] or daidzein-[4- ^{14}C], were incorporated into the

phytoalexins demethylhomopterocarpin, sativan and vestitol and also into 9-O-methylcoumestrol. The synthesis of 9-O-methylcoumestrol was greatly stimulated by this abiotic treatment, but coumestrol production was not noticeably affected. Daidzein and the trihydroxychalcone were precursors of coumestrol. A mechanism in which methylation is an integral part of the ***aryl*** migration process associated with the biosynthesis of 4'-methoxyisoflavonoids is possible. Formononetin, 2',7-dihydroxy-4'-methoxyisoflavone-[Me-14C], 7-hydroxy-4'-methoxyisoflavanone-[Me-14C] and 2',7-dihydroxy-4'-methoxyisoflavanone-[Me-14C] were all excellent precursors of demethylhomopterocarpin, sativan, vestitol and 9-O-methylcoumestrol; A metabolic grid may involved in their biosynthetic origin.

L1 ANSWER 3 OF 6 EMBASE COPYRIGHT 2004 ELSEVIER INC. ALL RIGHTS RESERVED.
on STN

ACCESSION NUMBER: 2001314407 EMBASE
TITLE: Lipase-catalyzed chemo- and enantioselective acetylation of 2-alkyl/ ***aryl*** -3-hydroxypropiophenones.
AUTHOR: Kumar R.; Azim A.; Kumar V.; Sharma S.K.; Prasad A.K.; Howarth O.W.; Olsen C.E.; Jain S.C.; Parmar V.S.
CORPORATE SOURCE: V.S. Parmar, Department of Chemistry, University of Delhi, Delhi-110 007, India. minuashok@now-india.net.in
SOURCE: Bioorganic and Medicinal Chemistry, (2001) 9/10 (2643-2652).
Refs: 29
ISSN: 0968-0896 CODEN: BMECEP
PUBLISHER IDENT.: S 0968-0896(01)00184-5
COUNTRY: United Kingdom
DOCUMENT TYPE: Journal; Conference Article
FILE SEGMENT: 029 Clinical Biochemistry
LANGUAGE: English
SUMMARY LANGUAGE: English

AB The chemo- and enantioselective capabilities of porcine pancreatic lipase (PPL) in tetrahydrofuran, and Candida rugosa lipase (CRL) in diisopropyl ether have been investigated for the acetylation of racemic 2-alkyl/ ***aryl*** -3-hydroxypropiophenones, which are important precursors in the synthesis of biologically active chromanones and ***isoflavanones***. A highly chemoselective acetylation of primary hydroxy group in preference to phenolic hydroxy group leading to the formation of enantiomerically enriched monoacetates has been observed. .COPYRGT. 2001 Elsevier Science Ltd. All rights reserved.

L1 ANSWER 6 OF 6 CAPLUS COPYRIGHT 2004 ACS on STN

ACCESSION NUMBER: 2000:646149 CAPLUS
DOCUMENT NUMBER: 133:249928
TITLE: Cytochromes P450 involved in isoflavone biosynthesis of soybean and Medicago and the genes encoding them and their uses
INVENTOR(S): Steele, Christopher L.; Dixon, Richard A.
PATENT ASSIGNEE(S): Samuel Roberts Noble Foundation, Inc., USA
SOURCE: PCT Int. Appl., 74 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

| PATENT NO. | KIND | DATE | APPLICATION NO. | DATE |
|--|--|----------|-----------------|------------|
| WO 2000053771 | A1 | 20000914 | WO 2000-US5915 | 20000308 |
| W: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM RW: GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG | | | | |
| AU 2000037287 | A5 | 20000928 | AU 2000-37287 | 20000308 |
| NZ 513992 | A | 20010928 | NZ 2000-513992 | 20000308 |
| EP 1161540 | A1 | 20011212 | EP 2000-916134 | 20000308 |
| R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO | | | | |
| PRIORITY APPLN. INFO.: | | | US 1999-123267P | P 19990308 |
| | | | WO 2000-US5915 | W 20000308 |
| AB | Soybean and Medicago truncatula CYP93C genes that encode a cytochrome P 450 that can catalyze the ***aryl*** migration of a ***flavanone*** to yield an ***isoflavanone*** intermediate or an isoflavone have been cloned. Plants can now be genetically engineered to produce isoflavones that provide potential human health benefits and increase disease resistance in plants. Isoflavones can now be produced in transgenic plants species in which isoflavones do not naturally occur, i.e., in species other than legumes. Alternatively, introducing infection-inducible isoflavonoid biosynthesis into non-legumes qual. complements these plants' phytoalexin defenses against microbial pathogens, whereas over-expression of the isoflavonoid pathway in legumes quant. increases this defense response. Finally, modifying the extend of prodn. of isoflavonoids in legume roots pos. impacts nodulation efficiency and therefore plant yield. | | | |
| REFERENCE COUNT: 13 THERE ARE 13 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT | | | | |
| => s aryl and flavanone and isoflavone | | | | |
| L2 | 41 ARYL AND FLAVANONE AND ISOFLAVONE | | | |
| => s flavanone and (isoflavone or isoflavanone) | | | | |
| L3 | 663 FLAVANONE AND (ISOFLAVONE OR ISOFLAVANONE) | | | |
| => s l3 and transform? | | | | |
| L4 | 28 L3 AND TRANSFORM? | | | |
| => s l4 and plant | | | | |
| L5 | 12 L4 AND PLANT | | | |
| => s l5 and aryl | | | | |
| L6 | 2 L5 AND ARYL | | | |
| => duplicate remove l5 | | | | |
| DUPLICATE PREFERENCE IS 'BIOSIS, EMBASE, CAPLUS' | | | | |
| KEEP DUPLICATES FROM MORE THAN ONE FILE? Y/(N):n | | | | |
| PROCESSING COMPLETED FOR L5 | | | | |
| L7 | 10 DUPLICATE REMOVE L5 (2 DUPLICATES REMOVED) | | | |

of AhR induced by 1 nM 2,3,7,8-tetrachlorodibenzo-p-dioxin without agonistic effects that ***transform*** AhR. The flavonoid IC50 values ranged 0.14-10 .mu.M, close to physiol. levels in man.

REFERENCE COUNT: 28 THERE ARE 28 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

=> d 15 1-10 ibib ab

L5 ANSWER 1 OF 12 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN
ACCESSION NUMBER: 2003:439741 BIOSIS
DOCUMENT NUMBER: PREV200300439741
TITLE: Cloning and expression of the ***isoflavone*** synthase gene (IFS-Tp) from Trifolium pratense.
AUTHOR(S): Kim, Bong Gyu; Kim, Song-Young; Song, Hee Su; Lee, Chan; Hur, Hor-Gil; Kim, Su-Il; Ahn, Joong-Hoon [Reprint Author]
CORPORATE SOURCE: Department of Forest and Environmental Science, Bio/Molecular Informatics Center, Konkuk University, Seoul, 143-701, South Korea
jhahn@konkuk.ac.kr
SOURCE: Molecules and Cells, (June 30 2003) Vol. 15, No. 3, pp. 301-306. print.
ISSN: 1016-8478 (ISSN print).
DOCUMENT TYPE: Article
LANGUAGE: English
OTHER SOURCE: DDBJ-AB023636; EMBL-AB023636; GenBank-AB023636;
DDBJ-AF195798; EMBL-AF195798; GenBank-AF195798;
DDBJ-AF195799; EMBL-AF195799; GenBank-AF195799;
DDBJ-AF195806; EMBL-AF195806; GenBank-AF195806;
DDBJ-AF195807; EMBL-AF195807; GenBank-AF195807;
DDBJ-AF195809; EMBL-AF195809; GenBank-AF195809;
DDBJ-AF462633; EMBL-AF462633; GenBank-AF462633;
DDBJ-AJ243804; EMBL-AJ243804; GenBank-AJ243804;
DDBJ-AY167424; EMBL-AY167424; GenBank-AY167424;
DDBJ-AY253284; EMBL-AY253284; GenBank-AY253284
ENTRY DATE: Entered STN: 24 Sep 2003
Last Updated on STN: 24 Sep 2003

AB ***Isoflavones*** are secondary metabolites found mainly in leguminous ***plants***. Their synthesis from ***flavanones*** is catalyzed by ***isoflavone*** synthase (IFS). We have cloned a ***isoflavone*** synthase gene (IFS-Tp) from Trifolium pratense that encodes a predicted 525 amino acids protein, molecular weight 59 kDa, with strong homology to IFS's from other legumes. IFS-Tp was expressed in all the tissues examined, and addition of glutathione and UV irradiation enhanced its expression. Microsomes from yeast ***transformed*** with IFS-Tp were able to convert naringenin to genistein, indicating that IFS-Tp has ***isoflavone*** synthase activity.

L5 ANSWER 2 OF 12 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN
ACCESSION NUMBER: 1991:314867 BIOSIS
DOCUMENT NUMBER: PREV199192025382; BA92:25382
TITLE: FLAVONOID 5-GLUCOSIDES FROM PRUNUS-CERASUS BARK AND THEIR CHARACTERISTIC WEAK GLYCOSIDIC BONDING.
AUTHOR(S): GEIBEL M [Reprint author]; FEUCHT W
CORPORATE SOURCE: INST PLANZENBAU, LEHRSTUHL OBSTBAU, TECHNISCHE UNIV MUENCHEN, D8050 FREISING-WEIHENSTEPHAN, FRG

SOURCE: Phytochemistry (Oxford), (1991) Vol. 30, No. 5, pp.
1519-1522.
CODEN: PYTCAS. ISSN: 0031-9422.

DOCUMENT TYPE: Article

FILE SEGMENT: BA

LANGUAGE: ENGLISH

ENTRY DATE: Entered STN: 15 Jul 1991

Last Updated on STN: 11 Sep 1991

AB Pinostrobin 5-glucoside, a novel ***flavanone*** glycoside, was isolated from the bark of *Prunus cerasus*. As it was not found in *P. avium*, the substance is useful to distinguish these two species. Apigenin 5-glucoside genkwanin 5-glucoside and neosakuranin were also isolated from the bark of *P. cerasus*. They occur in both species as minor components. These 5-glucosides together with genistein 5-glucoside, prunetin 5-glucoside, sakuranin, tectochrysin 5-glucoside and luteolin 5-glucoside were hydrolysed in malic acid. The ***isoflavone*** and flavone 5-glucosides were shown to be hydrolysed more rapidly than the ***flavanone*** 5-glucosides, whereas no hydrolysis was observed with the corresponding 7-glucosides under the same conditions. The chalcone 2'-glucoside neosakuranin was ***transformed*** at first to the corresponding flavonone 5-glucoside, which was hydrolysed thereafter.

L5 ANSWER 3 OF 12 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN

ACCESSION NUMBER: 1990:473176 BIOSIS

DOCUMENT NUMBER: PREV199090112596; BA90:112596

TITLE: MICROBIOLOGICAL ***TRANSFORMATION*** OF RACEMIC
FLAVANONE AND RACEMIC ***ISOFLAVANONE***.

AUTHOR(S): IBRAHIM A-R [Reprint author]; ABUL-HAJJ Y J

CORPORATE SOURCE: DEP MED CHEM, COLL OF PHARMACY, UNIV MINNESOTA,
MINNEAPOLIS, MINN 55455, USA

SOURCE: Journal of Natural Products (Lloydia), (1990) Vol. 53, No.
3, pp. 644-656.

CODEN: JNPRDF. ISSN: 0163-3864.

DOCUMENT TYPE: Article

FILE SEGMENT: BA

LANGUAGE: ENGLISH

ENTRY DATE: Entered STN: 25 Oct 1990

Last Updated on STN: 4 Jan 1991

AB The microbiological ***transformation*** of ***flavanone*** and ***isoflavanone*** was explored using a group of 80 microorganisms in the initial screening. Ten metabolites of ***flavanone*** were isolated and identified as 4'-hydroxyflavanone [3], 3',4'-dihydroxyflavone [4], 3-hydroxyflavone [2], ***flavanone*** [5], 2'-hydroxydihydrochalcone [7], 2',4-dihydroxydihydrochalcone [6], 2',3,4-trihydroxydihydrochalcone [8], 2',5'-dihydroxydihydrochalcone [9], 4'-hydroxyflavan-4.alpha.-ol [11], and 2'-hydroxydibenzoylmethane [10]. The ***isoflavanone*** metabolites were identified as ***isoflavone*** [15], 2-hydroxyisoflavanone [16], 4'-hydroxyisoflavanone [13], 6,4'-dihydroxyisoflavanone [17], and 3',4'-dihydroxyisoflavone [14]. The structures of the metabolites were established using spectroscopic techniques including ir, ms, uv, 1H-nmr, and 13C-nmr spectroscopy. Production of 4'-hydroxyflavanone, 3',4'-dihydroxyflavanone, and 2',4-dihydroxydihydrochalcone by 13 microorganisms was assayed using reversed-phase hplc.

L5 ANSWER 4 OF 12 EMBASE COPYRIGHT 2004 ELSEVIER INC. ALL RIGHTS RESERVED.
on STN

046 Environmental Health and Pollution Control
 052 Toxicology
 LANGUAGE: English
 SUMMARY LANGUAGE: English
 AB Dioxins invade the body mainly through the diet, and produce toxicity through the ***transformation*** of aryl hydrocarbon receptor (AhR). An inhibitor of the ***transformation*** should therefore protect against the toxicity and ideally be part of the diet. We examined flavonoids ubiquitously expressed in ***plant*** foods as one of the best candidates, and found that the subclasses flavones and flavonols suppressed antagonistically the ***transformation*** of AhR induced by 1 nM of 2,3,7,8-tetrachlorodibenzo-p-dioxin, without exhibiting agonistic effects that ***transform*** AhR. The antagonistic IC50 values ranged from 0.14 to 10 .mu.M, close to the physiological levels in human.
 Copyright (C) 2000 Federation of European Biochemical Societies.

L5 ANSWER 6 OF 12 CAPLUS COPYRIGHT 2004 ACS on STN
 ACCESSION NUMBER: 2004:252297 CAPLUS
 DOCUMENT NUMBER: 140:282425
 TITLE: Biosynthetic production of flavonoid and isoflavonoid nutraceuticals by genetic manipulation of enzymes in ***plants***
 INVENTOR(S): Dixon, Richard A.; Liu, Chang-jun; Deavours, Bettina
 PATENT ASSIGNEE(S): The Samuel Roberts Noble Foundation, Inc., USA
 SOURCE: PCT Int. Appl., 80 pp.
 CODEN: PIXXD2
 DOCUMENT TYPE: Patent
 LANGUAGE: English
 FAMILY ACC. NUM. COUNT: 1
 PATENT INFORMATION:

| PATENT NO. | KIND | DATE | APPLICATION NO. | DATE |
|---|------|----------|-----------------|----------|
| WO 2004024079 | A2 | 20040325 | WO 2003-US28454 | 20030910 |
| W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW, AM, AZ, BY, KG, KZ, MD RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PT, RO, SE, SI, SK, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG | | | | |

PRIORITY APPLN. INFO.: US 2002-409447P P 20020910
 AB The invention provides method and compns. for the modulation of ***flavanone*** and/or ***isoflavone*** prodn. in ***plants***. Transgenic ***plants*** having novel phenotypes are generated by down-regulating ***flavanone*** 3-hydroxylase by loss-of-function mutations or antisense oligonucleotides, and up-regulation of ***isoflavone*** synthase, chalcone isomerase, and/or chalcone synthase by cloning their genes into the recipient ***plants***. Introduction of ***isoflavone*** synthase into ***plants*** in which the ***flavanone*** 3-hydroxylase is down-regulated results in significant improvement to the levels of accumulation of the ***isoflavone*** genistein; this can be coupled with increasing flux into the prodn. of

naringenin to result in even further enhancement of ***isoflavone***
 prodn. Increased expression of ***isoflavones*** in particular in
 plants may be used to increase the nutritional value of food
 plants for both human and animal consumption. The invention
 overcomes limitations of the prior art which prevented accumulation of
 high levels of ***isoflavones*** in ***plants***.

L5 ANSWER 7 OF 12 CAPLUS COPYRIGHT 2004 ACS on STN

ACCESSION NUMBER: 2003:1007113 CAPLUS

DOCUMENT NUMBER: 140:56623

TITLE: Method to increase isoflavonoid levels through genetic
 engineering to modulate to gene expression in
 phenylpropanoid biosynthetic pathway in transgenic
 plants

INVENTOR(S): McGonigle, Brian; Odell, Joan T.

PATENT ASSIGNEE(S): E. I. Du Pont de Nemours & Co., USA

SOURCE: PCT Int. Appl., 55 pp.

CODEN: PIXXD2

DOCUMENT TYPE: Patent

LANGUAGE: English

FAMILY ACC. NUM. COUNT: 1

PATENT INFORMATION:

| PATENT NO. | KIND | DATE | APPLICATION NO. | DATE |
|------------|------|------|-----------------|------|
|------------|------|------|-----------------|------|

| | | | | |
|---------------|----|----------|-----------------|----------|
| WO 2003106633 | A2 | 20031224 | WO 2003-US18663 | 20030612 |
|---------------|----|----------|-----------------|----------|

W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN,
 CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH,
 GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR,
 LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NI, NO, NZ, OM,
 PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, TJ, TM, TN, TR, TT,
 TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW, AM, AZ, BY, KG, KZ,
 MD, RU, TJ, TM

RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW, AT, BE, BG,
 CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC,
 NL, PT, RO, SE, SI, SK, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ,
 GW, ML, MR, NE, SN, TD, TG

| | | | | |
|---------------|----|----------|----------------|----------|
| US 2004006795 | A1 | 20040108 | US 2003-459159 | 20030611 |
|---------------|----|----------|----------------|----------|

PRIORITY APPLN. INFO.: US 2002-388280P P 20020613

AB This invention pertains to methods of increasing isoflavonoid prodn. in
 isoflavonoid-producing ***plants*** by ***transforming***
 plants with at least one construct expressing at least a portion
 of a ***flavanone*** 3-hydroxylase, a C1 myb transcription factor, and
 an R-type myc transcription factor that regulate expression of genes in
 the phenylpropanoid pathway. Specifically, ***isoflavone*** levels in
 Glycine max (soybean) are increased via metabolic engineering of the
 complex phenylpropanoid biosynthetic pathway through suppression of
 flavanone 3-hydroxylase (F3H) to block the anthocyanin branch of
 the pathway, in combination with expressing C1/R fusion protein CRC to
 activate other related gene expression. The F3H suppression vector AC21
 contains a portion of FSH gene (antisense presumably, not specified, under
 the control of a seed-specific promoter) that can promote formation of a
 stem loop structure and thus inhibit F3H gene expression. The CRC vector
 (pOY135) encodes a fusion protein (under the control of phaseolin
 promoter) which contain corn C1 myb domain to amino acid 125, the entire
 coding region of the Lc allele of R, and C1 transcription activation
 domain (from amino acid 126 to the C-terminus of C1). Higher levels of

isoflavones (4-times than wild-type), and decreased genistein and increased the daidzein levels are detected in transgenic soybean seed.

L5 ANSWER 8 OF 12 CAPLUS COPYRIGHT 2004 ACS on STN

ACCESSION NUMBER: 2003:613490 CAPLUS

DOCUMENT NUMBER: 139:273742

TITLE: Cloning and expression of the ***isoflavone***
synthase gene (IFS-Tp) from Trifolium pratense

AUTHOR(S): Kim, Bong Gyu; Kim, Song-Young; Song, Hee Su; Lee,
Chan; Hur, Hor-Gil; Kim, Su-Il; Ahn, Joong-Hoon

CORPORATE SOURCE: Department of Forest and Environmental Science,
Bio/Molecular Informatics Center, KonKuk University,
Seoul, 143-701, S. Korea

SOURCE: Molecules and Cells (2003), 15(3), 301-306
CODEN: MOCEEK; ISSN: 1016-8478

PUBLISHER: Korean Society for Molecular and Cellular Biology

DOCUMENT TYPE: Journal

LANGUAGE: English

AB ***Isoflavones*** are secondary metabolites found mainly in leguminous
plants. Their synthesis from ***flavanones*** is catalyzed
by

isoflavone synthase (IFS). We have cloned a ***isoflavone***
synthase gene (IFS-Tp) from Trifolium pratense that encodes a predicted
525 amino acids protein, mol. wt. 59 kDa, with strong homol. to IFS's from
other legumes. IFS-Tp was expressed in all the tissues examd., and addn.
of glutathione and UV irradiation enhanced its expression. Microsomes from
yeast ***transformed*** with IFS-Tp were able to convert naringenin to
genistein, indicating that IFS-Tp has ***isoflavone*** synthase
activity.

REFERENCE COUNT: 22 THERE ARE 22 CITED REFERENCES AVAILABLE FOR THIS
RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L5 ANSWER 9 OF 12 CAPLUS COPYRIGHT 2004 ACS on STN

ACCESSION NUMBER: 2003:563752 CAPLUS

DOCUMENT NUMBER: 139:258149

TITLE: Metabolic engineering to increase ***isoflavone***
biosynthesis in soybean seed

AUTHOR(S): Yu, Oliver; Shi, June; Hession, Aideen O.; Maxwell,
Carl A.; McGonigle, Brian; Odell, Joan T.

CORPORATE SOURCE: Experimental Station, Crop Genetics, E.I. du Pont de
Nemours & Company, Inc., Wilmington, DE, 19880-0402,
USA

SOURCE: Phytochemistry (Elsevier) (2003), 63(7), 753-763
CODEN: PYTCAS; ISSN: 0031-9422

PUBLISHER: Elsevier Science B.V.

DOCUMENT TYPE: Journal

LANGUAGE: English

AB ***Isoflavone*** levels in Glycine max (soybean) were increased via
metabolic engineering of the complex phenylpropanoid biosynthetic pathway.
Phenylpropanoid pathway genes were activated by expression of the maize C1
and R transcription factors in soybean seed, which decreased genistein and
increased the daidzein levels with a small overall increase in total
isoflavone levels. Cosuppression of ***flavanone***
3-hydroxylase to block the anthocyanin branch of the pathway, in
conjunction with C1/R expression, resulted in higher levels of
isoflavones. The combination of transcription factor-driven gene
activation and suppression of a competing pathway provided a successful

L5 ANSWER 12 OF 12 CAPLUS COPYRIGHT 2004 ACS on STN
AN 2000:453735 CAPLUS
DN 133:222001
TI Flavones and flavonols at dietary levels inhibit a ***transformation***
of aryl hydrocarbon receptor induced by dioxin
AU Ashida, Hitoshi; Fukuda, Itsuko; Yamashita, Takatoshi; Kanazawa, Kazuki
CS Faculty of Agriculture, Laboratory of Food and Nutritional Chemistry, Kobe
University, Kobe, 657-8501, Japan
SO FEBS Letters (2000), 476(3), 213-217
CODEN: FEBLAL; ISSN: 0014-5793
PB Elsevier Science B.V.
DT Journal
LA English
RE.CNT 28 THERE ARE 28 CITED REFERENCES AVAILABLE FOR THIS RECORD
ALL CITATIONS AVAILABLE IN THE RE FORMAT

=> s cyp93c and function and P450
L8 0 CYP93C AND FUNCTION AND P450

=> s cyp93c and function
L9 0 CYP93C AND FUNCTION

=> s cyp93c
L10 8 CYP93C

=> duplicate remove l10
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PROCESSING COMPLETED FOR L10
L11 4 DUPLICATE REMOVE L10 (4 DUPLICATES REMOVED)

=> d l11 1-4 ibib ab

L11 ANSWER 1 OF 4 AGRICOLA Compiled and distributed by the National
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(2004) on STN DUPLICATE 1

ACCESSION NUMBER: 2003:46173 AGRICOLA
DOCUMENT NUMBER: IND23332870
TITLE: Key amino acid residues required for aryl migration
catalysed by the cytochrome P450 2-hydroxyisoflavanone
synthase.
AUTHOR(S): Sawada, Y.; Kinoshita, K.; Akashi, T.; Aoki, T.;
Ayabe, S.
AVAILABILITY: DNAL (QK710.P68)
SOURCE: The Plant journal : for cell and molecular biology,
Sept 2002. Vol. 31, No. 5. p. 555-564
Publisher: Oxford : Blackwell Sciences Ltd.
ISSN: 0960-7412
NOTE: Includes references
PUB. COUNTRY: England; United Kingdom
DOCUMENT TYPE: Article
FILE SEGMENT: Non-U.S. Imprint other than FAO
LANGUAGE: English
AB Isoflavonoids are distributed predominantly in leguminous plants, and play
pivotal roles in the interaction of host plants with biological

environments. Isoflavones in the diet also have beneficial effects on human health as phytoestrogens. The isoflavonoid skeleton is constructed by the ***CYP93C*** subfamily of cytochrome P450s in plant cells. The reaction consists of hydroxylation of the flavanone molecule at C-2 and an intramolecular 1,2-aryl migration from C-2 to C-3 to yield 2-hydroxyisoflavanone. In this study, with the aid of alignment of amino acid sequences of CYP93 family P450s and a computer-generated putative stereo structure of the protein, candidates for key amino acid residues in CYP93C2 responsible for the unique aryl migration in 2-hydroxyisoflavanone synthase reaction were identified. Microsomes of recombinant yeast cells expressing mutant proteins of CYP93C2 were prepared, and their catalytic activities tested. The reaction with the mutant in which Ser 310 in the centre of the I-helix was converted to Thr yielded increased formation of 3-hydroxyflavanone, a by-product of the 2-hydroxyisoflavanone synthase reaction, in addition to the major isoflavonoid product. More dramatically, the mutant in which Lys 375 in the end of beta-sheet 1-4 was replaced with Thr produced only 3-hydroxyflavanone and did not yield the isoflavonoid any longer. The roles of these amino acid residues in the catalysis and evolution of isoflavonoid biosynthesis are discussed.

L11 ANSWER 2 OF 4 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN
DUPLICATE 2

ACCESSION NUMBER: 2001:198852 BIOSIS
DOCUMENT NUMBER: PREV200100198852
TITLE: Cytochrome P450s as genes for crop improvement.
AUTHOR(S): Feldmann, Kenneth A. [Reprint author]
CORPORATE SOURCE: Ceres, Inc., 3007 Malibu Canyon Road, Malibu, CA, 90265,
USA
kfeldmann@ceres-inc.com
SOURCE: Current Opinion in Plant Biology, (April, 2001) Vol. 4, No.
2, pp. 162-167. print.
ISSN: 1369-5266.
DOCUMENT TYPE: Article
General Review; (Literature Review)
LANGUAGE: English
ENTRY DATE: Entered STN: 25 Apr 2001
Last Updated on STN: 18 Feb 2002

AB In the past year, several cytochrome P450 genes have been identified that will be important for generating crop protectants and natural medicinal products. Among these are the 2-hydroxyisoflavone synthase (***CYP93C***) and the indole-3-acetaldoxime N-hydroxylase (CYP83B1) genes, which catalyze the formation of isoflavones and glucosinolates, respectively.

L11 ANSWER 3 OF 4 CAPLUS COPYRIGHT 2004 ACS on STN

ACCESSION NUMBER: 2000:646149 CAPLUS
DOCUMENT NUMBER: 133:249928
TITLE: Cytochromes P450 involved in isoflavone biosynthesis of soybean and Medicago and the genes encoding them and their uses
INVENTOR(S): Steele, Christopher L.; Dixon, Richard A.
PATENT ASSIGNEE(S): Samuel Roberts Noble Foundation, Inc., USA
SOURCE: PCT Int. Appl., 74 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1

PATENT INFORMATION:

| PATENT NO. | KIND | DATE | APPLICATION NO. | DATE |
|--|---|---|-----------------|------------|
| ----- | ---- | ----- | ----- | ----- |
| WO 2000053771 | A1 | 20000914 | WO 2000-US5915 | 20000308 |
| W: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM RW: GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG | | | | |
| AU 2000037287 | A5 | 20000928 | AU 2000-37287 | 20000308 |
| NZ 513992 | A | 20010928 | NZ 2000-513992 | 20000308 |
| EP 1161540 | A1 | 20011212 | EP 2000-916134 | 20000308 |
| R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO | | | | |
| PRIORITY APPLN. INFO.: | | | US 1999-123267P | P 19990308 |
| | | | WO 2000-US5915 | W 20000308 |
| AB | Soybean and Medicago truncatula ***CYP93C*** genes that encode a cytochrome P 450 that can catalyze the aryl migration of a flavanone to yield an isoflavanone intermediate or an isoflavone have been cloned. Plants can now be genetically engineered to produce isoflavones that provide potential human health benefits and increase disease resistance in plants. Isoflavones can now be produced in transgenic plants species in which isoflavones do not naturally occur, i.e., in species other than legumes. Alternatively, introducing infection-inducible isoflavonoid biosynthesis into non-legumes qual. complements these plants' phytoalexin defenses against microbial pathogens, whereas over-expression of the isoflavonoid pathway in legumes quant. increases this defense response. Finally, modifying the extend of prodn. of isoflavonoids in legume roots pos. impacts nodulation efficiency and therefore plant yield. | | | |
| REFERENCE COUNT: | 13 | THERE ARE 13 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT | | |
| L11 | ANSWER 4 OF 4 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN | | | |
| | DUPLICATE 3 | | | |
| ACCESSION NUMBER: | 2001:151075 BIOSIS | | | |
| DOCUMENT NUMBER: | PREV200100151075 | | | |
| TITLE: | Induction of isoflavonoid pathway in the model legume Lotus japonicus: Molecular characterization of enzymes involved in phytoalexin biosynthesis. | | | |
| AUTHOR(S): | Shimada, Norimoto; Akashi, Tomoyoshi; Aoki, Toshio; Ayabe, Shin-ichi [Reprint author] | | | |
| CORPORATE SOURCE: | Department of Applied Biological Sciences, Nihon University, Fujisawa, Kanagawa, 252-8510, Japan ayabe@brs.nihon-u.ac.jp | | | |
| SOURCE: | Plant Science (Shannon), (December 7th, 2000) Vol. 160, No. 1, pp. 37-47. print. CODEN: PLSCE4. ISSN: 0168-9452. | | | |
| DOCUMENT TYPE: | Article | | | |
| LANGUAGE: | English | | | |
| ENTRY DATE: | Entered STN: 28 Mar 2001 Last Updated on STN: 15 Feb 2002 | | | |
| AB | Treatment of the seedlings of Lotus japonicus, a model legume for | | | |

molecular genetic studies, with reduced glutathione (GSH) resulted in the accumulation of an isoflavan phytoalexin, vestitol. Using PCR strategies based on the conserved amino acid sequences, full length P450 cDNAs were obtained from GSH-treated seedling roots. When the clones, LjCYP-1 (***CYP93C*** family) and LjCYP-2 (CYP81E family), were heterologously expressed in yeast, the proteins exhibited 2-hydroxyisoflavanone synthase (IFS) and isoflavone 2'-hydroxylase (I2'H) activities, respectively. The transcription levels of LjCYP-1, LjCYP-2 and isoflavone reductase, which are all involved in vestitol biosynthesis, coordinately increased upon elicitation. Genomic Southern blot analysis indicated that the IFS gene forms a small gene family and a single copy of the I2'H gene is present in the L. japonicus genome. Molecular biological aspects of P450s involved in the isoflavonoid pathway and the genomic approach to flavonoid metabolism in this unique plant are discussed.

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FILE CONTAINS CURRENT INFORMATION.
 LAST RELOADED: Jun 25, 2004 (20040625/UP).

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 47 FAMILY
 (FAMILY OR FAMILIES)
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| FULL ESTIMATED COST | 0.12 | 109.02 |
| DISCOUNT AMOUNTS (FOR QUALIFYING ACCOUNTS) | SINCE FILE | TOTAL |
| | ENTRY | SESSION |
| CA SUBSCRIBER PRICE | 0.00 | -5.88 |

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=> s cyp93c(w)family
L13 2 CYP93C(W) FAMILY

=> d l13 1-2

L13 ANSWER 1 OF 2 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN
AN 2001:151075 BIOSIS
DN PREV200100151075
TI Induction of isoflavonoid pathway in the model legume Lotus japonicus:
Molecular characterization of enzymes involved in phytoalexin
biosynthesis.
AU Shimada, Norimoto; Akashi, Tomoyoshi; Aoki, Toshio; Ayabe, Shin-ichi
[Reprint author]
CS Department of Applied Biological Sciences, Nihon University, Fujisawa,
Kanagawa, 252-8510, Japan
ayabe@brs.nihon-u.ac.jp
SO Plant Science (Shannon), (December 7th, 2000) Vol. 160, No. 1, pp. 37-47.
print.
CODEN: PLSCE4. ISSN: 0168-9452.
DT Article
LA English
ED Entered STN: 28 Mar 2001
Last Updated on STN: 15 Feb 2002

L13 ANSWER 2 OF 2 CAPLUS COPYRIGHT 2004 ACS on STN
AN 2001:74319 CAPLUS
DN 134:292777
TI Induction of isoflavonoid pathway in the model legume Lotus japonicus:
molecular characterization of enzymes involved in phytoalexin biosynthesis
AU Shimada, N.; Akashi, T.; Aoki, T.; Ayabe, S.-i.
CS Department of Applied Biological Sciences, Nihon University, Kanagawa,
Fujisawa, 252-8510, Japan
SO Plant Science (Shannon, Ireland) (2000), 160(1), 37-47
CODEN: PLSCE4; ISSN: 0168-9452
PB Elsevier Science Ireland Ltd.
DT Journal
LA English
RE.CNT 45 THERE ARE 45 CITED REFERENCES AVAILABLE FOR THIS RECORD
ALL CITATIONS AVAILABLE IN THE RE FORMAT

=> s cyp93c1
L14 3 CYP93C1

=> d l14 1-3 ti

L14 ANSWER 1 OF 3 CAPLUS COPYRIGHT 2004 ACS on STN
TI Plant nucleic acid sequences encoding isoflavone synthase

L14 ANSWER 2 OF 3 CAPLUS COPYRIGHT 2004 ACS on STN
TI Identification and expression of isoflavone synthase, the key enzyme for

biosynthesis of isoflavones in legumes

L14 ANSWER 3 OF 3 CAPLUS COPYRIGHT 2004 ACS on STN
TI Cytochrome P450 genes of soybean and their use in introduction of
phenylurea herbicide resistance in plants

=> d l14 1-3 ibib ab

L14 ANSWER 1 OF 3 CAPLUS COPYRIGHT 2004 ACS on STN
ACCESSION NUMBER: 2000:535288 CAPLUS
DOCUMENT NUMBER: 133:130818
TITLE: Plant nucleic acid sequences encoding isoflavone
synthase
INVENTOR(S): Fader, Gary M.; Jung, Woosuk; McGonigle, Brian; Odell,
Joan T.; Yu, Xiaodan
PATENT ASSIGNEE(S): E. I. Du Pont de Nemours & Co., USA
SOURCE: PCT Int. Appl., 157 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

| PATENT NO. | KIND | DATE | APPLICATION NO. | DATE |
|---|------|----------|-----------------|------------|
| WO 2000044909 | A1 | 20000803 | WO 2000-US1772 | 20000126 |
| W: AE, AL, AU, BA, BB, BG, BR, CA, CN, CR, CU, CZ, DM, EE, GD, GE, HR, HU, ID, IL, IN, IS, JP, KP, KR, LC, LK, LR, LT, LV, MG, MK, MN, MX, NO, NZ, PL, RO, SG, SI, SK, SL, TR, TT, UA, US, UZ, VN, YU, ZA, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM RW: GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG | | | | |
| EP 1147199 | A1 | 20011024 | EP 2000-907017 | 20000126 |
| R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO | | | | |
| PRIORITY APPLN. INFO.: | | | US 1999-117769P | P 19990127 |
| | | | US 1999-144783P | P 19990720 |
| | | | US 1999-156094P | P 19990924 |
| | | | WO 2000-US1772 | W 20000126 |
| AB This invention relates to isolated nucleic acid sequences encoding isoflavone synthase isolated from soybean, alfalfa, hairy vetch, lentil, mung bean, red clover, snow pea, white clover, sugar beet, and lupine. The invention also relates to the construction of chimeric sequences encoding all or a substantial portion of the enzymes, in sense or antisense orientation, wherein expression of the chimeric sequence results in prodn. of altered levels of the enzyme in a transformed host cell. Expression in such cells as yeast, tobacco, Arabidopsis thaliana, corn (monocot), and soybean result in altered levels of isoflavonoids (genestein, daidzein, naringenin, liquiritigenin). | | | | |
| REFERENCE COUNT: 8 THERE ARE 8 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT | | | | |

L14 ANSWER 2 OF 3 CAPLUS COPYRIGHT 2004 ACS on STN
ACCESSION NUMBER: 2000:107672 CAPLUS
DOCUMENT NUMBER: 132:247954

TITLE: Identification and expression of isoflavone synthase, the key enzyme for biosynthesis of isoflavones in legumes

AUTHOR(S): Jung, Woosuk; Yu, Oliver; Lau, Sze-Mei Cindy; O'Keefe, Daniel P.; Odell, Joan; Fader, Gary; McGonigle, Brian

CORPORATE SOURCE: Agricultural Biotechnology, The Dupont Company, Wilmington, DE, 19880-0402, USA

SOURCE: Nature Biotechnology (2000), 18(2), 208-212
CODEN: NABIF9; ISSN: 1087-0156

PUBLISHER: Nature America

DOCUMENT TYPE: Journal

LANGUAGE: English

AB Isoflavones have drawn much attention because of their benefits to human health. These compds., which are produced almost exclusively in legumes, have natural roles in plant defense and root nodulation. Isoflavone synthase catalyzes the first committed step of isoflavone biosynthesis, a branch of the phenyl-propanoid pathway. To identify the gene encoding this enzyme, we used a yeast expression assay to screen soybean ESTs encoding cytochrome P 450 proteins. We identified two soybean genes encoding isoflavone synthase, and used them to isolate homologous genes from other leguminous species including red clover, white clover, hairy vetch, mung bean, alfalfa, lentil, snow pea, and lupine, as well as from the nonleguminous sugar beet. We expressed soybean isoflavone synthase in Arabidopsis thaliana, which led to prodn. of the isoflavone genistein in this nonlegume plant. Identification of the isoflavone synthase gene should allow manipulation of the phenylpropanoid pathway for agronomic and nutritional purposes.

REFERENCE COUNT: 39 THERE ARE 39 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L14 ANSWER 3 OF 3 CAPLUS COPYRIGHT 2004 ACS on STN

ACCESSION NUMBER: 1999:271503 CAPLUS

DOCUMENT NUMBER: 130:292825

TITLE: Cytochrome P450 genes of soybean and their use in introduction of phenylurea herbicide resistance in plants

INVENTOR(S): Siminszky, Balazs; Dewey, Ralph E.; Corbin, Frederick T.

PATENT ASSIGNEE(S): North Carolina State University, USA

SOURCE: PCT Int. Appl., 92 pp.
CODEN: PIXXD2

DOCUMENT TYPE: Patent

LANGUAGE: English

FAMILY ACC. NUM. COUNT: 1

PATENT INFORMATION:

| PATENT NO. | KIND | DATE | APPLICATION NO. | DATE |
|---|------|----------|-----------------|----------|
| WO 9919493 | A2 | 19990422 | WO 1998-US20807 | 19981005 |
| WO 9919493 | A3 | 19990701 | | |
| W: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, CZ, DE, DE, DK, DK, EE, EE, ES, FI, FI, GB, GE, GH, GM, HR, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM | | | | |
| RW: GH, GM, KE, LS, MW, SD, SZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, | | | | |

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L2 ANSWER 1 OF 6 AGRICOLA Compiled and distributed by the National
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(2004) on STN DUPLICATE 1

ACCESSION NUMBER: 2003:46173 AGRICOLA

DOCUMENT NUMBER: IND23332870

TITLE: Key amino acid residues required for aryl migration
catalysed by the ***cytochrome*** ***P450***
2-hydroxyisoflavanone synthase.

AUTHOR(S): Sawada, Y.; Kinoshita, K.; Akashi, T.; Aoki, T.;
Ayabe, S.
AVAILABILITY: DNAL (QK710.P68)
SOURCE: The Plant journal : for cell and molecular biology,
Sept 2002. Vol. 31, No. 5. p. 555-564
Publisher: Oxford : Blackwell Sciences Ltd.
ISSN: 0960-7412
NOTE: Includes references
PUB. COUNTRY: England; United Kingdom
DOCUMENT TYPE: Article
FILE SEGMENT: Non-U.S. Imprint other than FAO
LANGUAGE: English

AB Isoflavonoids are distributed predominantly in leguminous plants, and play pivotal roles in the interaction of host plants with biological environments. Isoflavones in the diet also have beneficial effects on human health as phytoestrogens. The isoflavonoid skeleton is constructed by the CYP93C subfamily of ***cytochrome*** P450s in plant cells. The reaction consists of hydroxylation of the flavanone molecule at C-2 and an intramolecular 1,2-aryl migration from C-2 to C-3 to yield 2-hydroxyisoflavanone. In this study, with the aid of alignment of amino acid sequences of ***CYP93*** family P450s and a computer-generated putative stereo structure of the protein, candidates for key amino acid residues in CYP93C2 responsible for the unique aryl migration in 2-hydroxyisoflavanone synthase reaction were identified. Microsomes of recombinant yeast cells expressing mutant proteins of CYP93C2 were prepared, and their catalytic activities tested. The reaction with the mutant in which Ser 310 in the centre of the I-helix was converted to Thr yielded increased formation of 3-hydroxyflavanone, a by-product of the 2-hydroxyisoflavanone synthase reaction, in addition to the major isoflavonoid product. More dramatically, the mutant in which Lys 375 in the end of beta-sheet 1-4 was replaced with Thr produced only 3-hydroxyflavanone and did not yield the isoflavonoid any longer. The roles of these amino acid residues in the catalysis and evolution of isoflavonoid biosynthesis are discussed.

L2 ANSWER 2 OF 6 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN
DUPLICATE 2

ACCESSION NUMBER: 2000:324373 BIOSIS
DOCUMENT NUMBER: PREV200000324373
TITLE: Cloning and characterization of eight ***cytochrome***
P450 cDNAs from chickpea (*Cicer arietinum* L.) cell
suspension cultures.
AUTHOR(S): Overkamp, Stefan; Hein, Frauke; Barz, Wolfgang [Reprint
author]
CORPORATE SOURCE: Institut fuer Biochemie und Biotechnologie der Pflanzen,
Westfaelische Wilhelms-Universitaet Muenster,
Hindenburgplatz 55, 48143, Muenster, Germany
SOURCE: Plant Science (Shannon), (June 12th, 2000) Vol. 155, No. 1,
pp. 101-108. print.
CODEN: PLSCE4. ISSN: 0168-9452.
DOCUMENT TYPE: Article
LANGUAGE: English
ENTRY DATE: Entered STN: 2 Aug 2000
Last Updated on STN: 7 Jan 2002

AB Eight different ***P450*** sequences were isolated from a cDNA library derived from cultured chickpea cells (cultivar ILC3279) elicited with a *Phytophthora sojae* (formerly *megasperma*) elicitor (Pmg-elicitor) by

screening with heterologous and homologous probes. Screening with CYP73A1 from *Helianthus tuberosus* yielded several clones with one identical sequence. A full-length clone could be isolated and this sequence was assigned CYP73A19. Heterologous expression in yeast confirmed that CYP73A19 is the trans-cinnamic acid 4-hydroxylase of chickpea. Screening with a CYP81E2 polymerase chain reaction fragment from chickpea yielded a CYP81E2 full-length sequence and two almost identical CYP81E3 sequences, differing in only 16 out of 498 amino acids; both share more than 85% homology with the isoflavone 2'-hydroxylase from licorice (*Glycyrrhiza echinata* L.). Using CYP93A1 as a probe, it was possible to isolate a full-length member of the ***CYP93*** family, CYP93C3, that shares more than 80% homology with isoflavone synthase from soybean. In addition, partial sequences CYP81E3, CYP81E4 and CYP81E5 were also found in this screening. The use of a CYP82A2 probe derived from BAC F10N7 from *Arabidopsis thaliana* yielded only one sequence, CYP76F1. Rescreening with CYP81E4 and CYP81E5 did not result in the isolation of any new ***P450*** sequences. Northern blot experiments revealed that all but the CYP76F1 are induced rapidly and transiently in cell cultures upon elicitor treatment.

L2 ANSWER 3 OF 6 CAPLUS COPYRIGHT 2004 ACS on STN

ACCESSION NUMBER: 2000:24762 CAPLUS
DOCUMENT NUMBER: 132:332077
TITLE: Functional analysis of a novel jasmonate-inducible
cytochrome ***P450***, CYP93A1, in
soybean: the involvement in isoflavone biosynthesis
and induction by a fungal elicitor
AUTHOR(S): Ohta, Hiroyuki; Sueoka, Hideaki; Suzuki, Genki;
Takamiya, Ken-Ichiro
CORPORATE SOURCE: Faculty of Bioscience and Biotechnology, Tokyo
Institute of Technology, Yokohama, 226-8501, Japan
SOURCE: Daizu Tanpakushitsu Kenkyu (1999), 2, 1-4
CODEN: DTKEFV; ISSN: 1344-4050
PUBLISHER: Fuji Tanpakushitsu Kenkyu Shinko Zaidan
DOCUMENT TYPE: Journal
LANGUAGE: Japanese

AB We have isolated two cDNAs encoding novel jasmonate-inducible proteins from soybean suspension-cultured cells. One of the clone encodes a ***cytochrome*** P 450 belonging to a new family, ***CYP93*** (CYP93A1). CYP93A1 mRNA was also specifically induced in soybean cells by an elicitor which was derived from cell wall fraction of fungal pathogen, *Phytophthora megasperma* f. sp. *glycinea*. Furthermore, the mRNA was induced earlier than the accumulation of phytoalexin glyceollin in soybean cotyledon. These facts indicated that the induction of CYP93A1 mRNA is closely assocd. with the biosynthesis of glyceollin, a major phytoalexin in soybean cells. Thus, we analyzed the activities of (2S)-flavanone 2-hydroxylase and isoflavone synthase using CYP93A1 protein expressed in insect cells. However, no activity was obsd. Independently, Schopfer et al also analyzed the function of the CYP93A1, and found that the enzyme is dihydroxypterocarpan 6a-hydroxylase. We screened the genomic gene of ***CYP93*** family using 1.2 kb fragment of CYP93A1 cDNA, and obtained two different types of clones corresponding to CYP93A2 and CYP93A3.

L2 ANSWER 4 OF 6 CAPLUS COPYRIGHT 2004 ACS on STN

ACCESSION NUMBER: 1997:586978 CAPLUS
DOCUMENT NUMBER: 127:275617
TITLE: Cloning of cDNA for a novel methyl jasmonat-inducible

cytochrome ***P450*** (***CYP93***
 family) from the cultured soybean cells
 INVENTOR(S): Shibata, Daisuke; Kato, Tomohiko; Ota, Hiroyuki
 PATENT ASSIGNEE(S): Mitsui Gyosai Shokubutsu Bio Kenkyusho K. K., Japan
 SOURCE: Jpn. Kokai Tokkyo Koho, 10 pp.
 CODEN: JKXXAF
 DOCUMENT TYPE: Patent
 LANGUAGE: Japanese
 FAMILY ACC. NUM. COUNT: 1
 PATENT INFORMATION:

| PATENT NO. | KIND | DATE | APPLICATION NO. | DATE |
|------------------------|------|----------|-----------------|----------|
| ----- | ---- | ----- | ----- | ----- |
| JP 09224671 | A2 | 19970902 | JP 1996-31075 | 19960219 |
| PRIORITY APPLN. INFO.: | | | JP 1996-31075 | 19960219 |

AB The cDNA encoding a novel ***cytochrome*** P 450 (gene CYP93A1) from soybean suspension-cultured cells that had been treated with Me jasmonate (MeJA). The amino acid sequence of the gene product had 30-40% identity with those of other plant P450s. The protein contained the heme-binding domain which is highly conserved among plant P450s. Transcription of the
 cytochrome P 450 gene in soybean cells was induced by 30 .mu.M MeJA even in the presence of cycloheximide, and reached max. level 6 h after MeJA treatment. This is the first report of a plant
 cytochrome P 450 gene whose transcription is induced by MeJA even without protein synthesis.

L2 ANSWER 5 OF 6 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN
 DUPLICATE 3
 ACCESSION NUMBER: 1997:366779 BIOSIS
 DOCUMENT NUMBER: PREV199799658712
 TITLE: Cloning of ***cytochrome*** ***P450*** cDNAs from cultured Glycyrrhiza echinata L. cells and their transcriptional activation by elicitor-treatment.
 AUTHOR(S): Akashi, Tomoyoshi; Aoki, Toshio; Takahashi, Takeyoshi; Kameya, Nanako; Nakamura, Ikuo; Ayabe, Shin-Ichi [Reprint author]
 CORPORATE SOURCE: Dep. Applied Biological Science, Nihon Univ., Fujiwawa, Kanagawa 252, Japan
 SOURCE: Plant Science (Shannon), (1997) Vol. 126, No. 1, pp. 39-47.
 CODEN: PLSCE4. ISSN: 0168-9452.
 DOCUMENT TYPE: Article
 LANGUAGE: English
 ENTRY DATE: Entered STN: 25 Aug 1997
 Last Updated on STN: 25 Aug 1997

AB From a cDNA library of cultured Glycyrrhiza echinata L. (Fabaceae) cells treated with yeast extract (YE) elicitor, eight ***cytochrome***
 P450 (***P450***) fragments (Ge-1 to 8) were isolated using the PCR method based on conserved sequences of P450s. Comparison of amino acid sequences revealed that two of the fragments (Ge-1 and 2) belong to the CYP73 family encoding trans-cinnamic acid 4-hydroxylase. Ge-4 had 63% identity with the sequence included in ***CYP93*** , which has recently been reported to be induced by methyl jasmonate in soybean cells (Suzuki et al., FEBS Lett., 383 (1996) 83-86), and Ge-6 and 7 were highly homologous to the partial sequence of CYP84 encoding ferulic acid 5-hydroxylase of Arabidopsis thaliana (Meyer et al., Proc. Natl. Acad. Sci. USA, 93 (1996) 6869-6874). Others (Ge-3, 5 and 8) displayed homology less than 43% with known plant P450s. A full-length cDNA

(CYP73A14) containing Ge-1 sequence was isolated from the cDNA library, and its amino acid sequence showed 91-93% identity with CYP73 of other leguminous plant species. Northern blot analysis of mRNAs of *G. echinata* cells indicated that CYP73 (Ge-1 and 2) genes were transcribed at the early stages post-elicitation (lt 10 h). The transcription level of the Ge-1 gene was much higher than that of Ge-2. Transcription of Ge-3, 5, 6 and 7 was also activated by elicitation, but no clear signal from Ge-4 and 8 was observed. When total RNAs of cultured cells of *Medicago sativa* were analyzed by Northern blotting, transcripts hybridized with *G. echinata* cDNA clones containing Ge-1, 3, 5 and 6 sequences could be detected. Possible involvement of cloned P450s in elicitor-induced phenylpropanoid/flavonoid biosynthesis is discussed.

L2 ANSWER 6 OF 6 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN
DUPLICATE 4

ACCESSION NUMBER: 1996:187813 BIOSIS

DOCUMENT NUMBER: PREV199698743942

TITLE: Induction of a novel ***cytochrome*** ***P450*** (***CYP93*** family) by methyl jasmonate in soybean suspension-cultured cells.

AUTHOR(S): Suzuki, Genki; Ohta, Hiroyuki [Reprint author]; Kato, Tomohiko; Igarashi, Takao; Sakai, Fukumi; Shibata, Daisuke; Takano, Atuo; Masuda, Tatsuru; Shioi, Yuzo; Takamiya, Ken-Ichiro

CORPORATE SOURCE: Dep. Biol. Sci., Fac. Biosci. Biotechnol., Tokyo Inst. Technol., Nagatsuta, Midori-ku, Yokohama 226, Japan

SOURCE: FEBS Letters, (1996) Vol. 383, No. 1-2, pp. 83-86.

CODEN: FEBLAL. ISSN: 0014-5793.

DOCUMENT TYPE: Article

LANGUAGE: English

ENTRY DATE: Entered STN: 29 Apr 1996

Last Updated on STN: 10 Jun 1996

AB We isolated a cDNA encoding a novel ***cytochrome*** ***P450*** (CYP93A1) from soybean suspension-cultured cells that had been treated with methyl jasmonate (MeJA). The amino acid sequence of the gene product had 30-40% identity with those of other plant P450s. The protein contained the heme-binding domain which is highly conserved among plant P450s. Transcription of the ***cytochrome*** ***P450*** gene in soybean cells was induced by 30 μ -M MeJA even in the presence of cycloheximide, and reached maximum level 6 h after MeJA treatment. This is the first report of a plant ***cytochrome*** ***P450*** gene whose transcription is induced by MeJA even without protein synthesis.

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L3 8 CYP93C AND SYNTHASE

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DUPLICATE PREFERENCE IS 'AGRICOLA, BIOSIS, CAPLUS'

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PROCESSING COMPLETED FOR L3

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L4 ANSWER 1 OF 4 AGRICOLA Compiled and distributed by the National Agricultural Library of the Department of Agriculture of the United States

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DUPLICATE 1

ACCESSION NUMBER: 2003:46173 AGRICOLA
DOCUMENT NUMBER: IND23332870
TITLE: Key amino acid residues required for aryl migration
catalysed by the cytochrome P450 2-hydroxyisoflavanone
synthase
AUTHOR(S): Sawada, Y.; Kinoshita, K.; Akashi, T.; Aoki, T.;
Ayabe, S.
AVAILABILITY: DNAL (QK710.P68)
SOURCE: The Plant journal : for cell and molecular biology,
Sept 2002. Vol. 31, No. 5. p. 555-564
Publisher: Oxford : Blackwell Sciences Ltd.
ISSN: 0960-7412
NOTE: Includes references
PUB. COUNTRY: England; United Kingdom
DOCUMENT TYPE: Article
FILE SEGMENT: Non-U.S. Imprint other than FAO
LANGUAGE: English

AB Isoflavonoids are distributed predominantly in leguminous plants, and play pivotal roles in the interaction of host plants with biological environments. Isoflavones in the diet also have beneficial effects on human health as phytoestrogens. The isoflavonoid skeleton is constructed by the ***CYP93C*** subfamily of cytochrome P450s in plant cells. The reaction consists of hydroxylation of the flavanone molecule at C-2 and an intramolecular 1,2-aryl migration from C-2 to C-3 to yield 2-hydroxyisoflavanone. In this study, with the aid of alignment of amino acid sequences of CYP93 family P450s and a computer-generated putative stereo structure of the protein, candidates for key amino acid residues in CYP93C2 responsible for the unique aryl migration in 2-hydroxyisoflavanone ***synthase*** reaction were identified. Microsomes of recombinant yeast cells expressing mutant proteins of CYP93C2 were prepared, and their catalytic activities tested. The reaction with the mutant in which Ser 310 in the centre of the I-helix was converted to Thr yielded increased formation of 3-hydroxyflavanone, a by-product of the 2-hydroxyisoflavanone ***synthase*** reaction, in addition to the major isoflavonoid product. More dramatically, the mutant in which Lys 375 in the end of beta-sheet 1-4 was replaced with Thr produced only 3-hydroxyflavanone and did not yield the isoflavonoid any longer. The roles of these amino acid residues in the catalysis and evolution of isoflavonoid biosynthesis are discussed.

L4 ANSWER 2 OF 4 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN
DUPLICATE 2

ACCESSION NUMBER: 2001:198852 BIOSIS
DOCUMENT NUMBER: PREV200100198852
TITLE: Cytochrome P450s as genes for crop improvement.
AUTHOR(S): Feldmann, Kenneth A. [Reprint author]
CORPORATE SOURCE: Ceres, Inc., 3007 Malibu Canyon Road, Malibu, CA, 90265,
USA
kfeldmann@ceres-inc.com
SOURCE: Current Opinion in Plant Biology, (April, 2001) Vol. 4, No.
2, pp. 162-167. print.
ISSN: 1369-5266.
DOCUMENT TYPE: Article
General Review; (Literature Review)
LANGUAGE: English

ENTRY DATE: Entered STN: 25 Apr 2001
Last Updated on STN: 18 Feb 2002

AB In the past year, several cytochrome P450 genes have been identified that will be important for generating crop protectants and natural medicinal products. Among these are the 2-hydroxyisoflavone ***synthase*** (***CYP93C***) and the indole-3-acetaldoxime N-hydroxylase (CYP83B1) genes, which catalyze the formation of isoflavones and glucosinolates, respectively.

L4 ANSWER 3 OF 4 CAPLUS COPYRIGHT 2004 ACS on STN

ACCESSION NUMBER: 2000:646149 CAPLUS
DOCUMENT NUMBER: 133:249928
TITLE: Cytochromes P450 involved in isoflavone biosynthesis of soybean and Medicago and the genes encoding them and their uses
INVENTOR(S): Steele, Christopher L.; Dixon, Richard A.
PATENT ASSIGNEE(S): Samuel Roberts Noble Foundation, Inc., USA
SOURCE: PCT Int. Appl., 74 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

| PATENT NO. | KIND | DATE | APPLICATION NO. | DATE |
|---------------|--|----------|-----------------|----------|
| WO 2000053771 | A1 | 20000914 | WO 2000-US5915 | 20000308 |
| W: | AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM | | | |
| RW: | GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG | | | |
| AU 2000037287 | A5 | 20000928 | AU 2000-37287 | 20000308 |
| NZ 513992 | A | 20010928 | NZ 2000-513992 | 20000308 |
| EP 1161540 | A1 | 20011212 | EP 2000-916134 | 20000308 |
| R: | AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO | | | |

PRIORITY APPLN. INFO.: US 1999-123267P P 19990308
WO 2000-US5915 W 20000308

AB Soybean and Medicago truncatula ***CYP93C*** genes that encode a cytochrome P 450 that can catalyze the aryl migration of a flavanone to yield an isoflavanone intermediate or an isoflavone have been cloned. Plants can now be genetically engineered to produce isoflavones that provide potential human health benefits and increase disease resistance in plants. Isoflavones can now be produced in transgenic plants species in which isoflavones do not naturally occur, i.e., in species other than legumes. Alternatively, introducing infection-inducible isoflavonoid biosynthesis into non-legumes qual. complements these plants' phytoalexin defenses against microbial pathogens, whereas over-expression of the isoflavonoid pathway in legumes quant. increases this defense response. Finally, modifying the extend of prodn. of isoflavonoids in legume roots pos. impacts nodulation efficiency and therefore plant yield.

REFERENCE COUNT: 13 THERE ARE 13 CITED REFERENCES AVAILABLE FOR THIS

RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L4 ANSWER 4 OF 4 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN
DUPLICATE 3
ACCESSION NUMBER: 2001:151075 BIOSIS
DOCUMENT NUMBER: PREV200100151075
TITLE: Induction of isoflavonoid pathway in the model legume Lotus japonicus: Molecular characterization of enzymes involved in phytoalexin biosynthesis.
AUTHOR(S): Shimada, Norimoto; Akashi, Tomoyoshi; Aoki, Toshio; Ayabe, Shin-ichi [Reprint author]
CORPORATE SOURCE: Department of Applied Biological Sciences, Nihon University, Fujisawa, Kanagawa, 252-8510, Japan ayabe@brs.nihon-u.ac.jp
SOURCE: Plant Science (Shannon), (December 7th, 2000) Vol. 160, No. 1, pp. 37-47. print.
CODEN: PLSCE4. ISSN: 0168-9452.
DOCUMENT TYPE: Article
LANGUAGE: English
ENTRY DATE: Entered STN: 28 Mar 2001
Last Updated on STN: 15 Feb 2002

AB Treatment of the seedlings of Lotus japonicus, a model legume for molecular genetic studies, with reduced glutathione (GSH) resulted in the accumulation of an isoflavan phytoalexin, vestitol. Using PCR strategies based on the conserved amino acid sequences, full length P450 cDNAs were obtained from GSH-treated seedling roots. When the clones, LjCYP-1 (***CYP93C*** family) and LjCYP-2 (CYP81E family), were heterologously expressed in yeast, the proteins exhibited 2-hydroxyisoflavanone ***synthase*** (IFS) and isoflavone 2'-hydroxylase (12'H) activities, respectively. The transcription levels of LjCYP-1, LjCYP-2 and isoflavone reductase, which are all involved in vestitol biosynthesis, coordinately increased upon elicitation. Genomic Southern blot analysis indicated that the IFS gene forms a small gene family and a single copy of the 12'H gene is present in the L. japonicus genome. Molecular biological aspects of P450s involved in the isoflavonoid pathway and the genomic approach to flavonoid metabolism in this unique plant are discussed.

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